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A two-step process for biodiesel production from salmon oil

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Salmon oil, a by-product of salmon processing, was used as a feedstock for biodiesel production via transesterification in a two-step process. Two different types of salmon oil were tested: salmon oil extracted from acidified salmon hydrolysate and salmon oil extracted from salmon by-products. Optimal amounts of chemicals required to give the highest biodiesel yield from each oil were determined using batch production procedures. It was found that due to the high acid value of salmon oil, alkaline-catalysed transesterification was not an effective method for producing biodiesel from the salmon oil. Therefore a two-step process was applied, in which a sulphuric acid-catalysed pre-treatment was used in the first step to reduce the acid value from 12.0 to 3 mg [KOH] g [oil]^{−1} and then, in the second step, KOH-catalysed transesterification was applied. All experiments were performed at a temperature of 52±2 °C with a mixing intensity of 600 rpm. Based on the total weight of salmon oil used, the maximum biodiesel yield of 99% was achieved using a total methanol/molar ratio of 9.2% and 0.5% (w/w) KOH. Ester loss due to the formation of emulsion during the washing and drying steps was 15% maximum. This loss could be reduced in practical applications by better design of washing and drying techniques. A preliminary economic analysis showed that the cost of biodiesel production from salmon oil was almost twice that produced from soybean oil.

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1. Introduction

About 250,000 MT of fish processing waste is produced worldwide from salmon farming and could be converted into useful products such as salmon hydrolysate and oil (Wright, 2004). Kristinsson and Rasco (2000) reviewed different techniques for production of fish hydrolysate. The hydrolysate is typically produced by maceration of viscera or skinny by-products followed by enzymatic digestion of protein and

removal of bones. In the final stage, the hydrolysate is concentrated by either spray drying to produce a powder or evaporation to produce a thick liquid. Acid is normally added to stabilise the final product by lowering its pH to about 3.7. The hydrolysate contains about 10% fish oil. Oil can be separated from the acidified salmon hydrolysate or raw salmon by-products by centrifugation. Due to the acidification and sanitary handling of salmon by-products used for producing hydrolysates, this oil cannot be used for edible and

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pharmaceutical applications. Therefore, other uses for the oil, for example as a fuel, need to be explored. An estimated 65% of fish oil produced annually as a by-product of Alaskan seafood processing operations is consumed on-site as boiler fuel (Steigers, 2003). When directly used as fuel for engines either by itself or by blending it with other solvents, the fish oil could cause problems, such as carbon deposits in engine, engine durability and lubricating oil contamination (Ma & Hanna, 1999). Converting fish oil into biodiesel would be a better approach.

Biodiesel (fatty-acid alkyl esters) is a renewable and environmentally friendly energy source. It can be produced from plant oils and animal fats. Several techniques are available for biodiesel production. The most commonly used technique is transesterification in which triglycerides are reacted with alcohol, usually methanol, in the presence of a catalyst, usually potassium or sodium hydroxide (KOH or NaOH), to produce mono alkyl esters. Many factors affect the biodiesel yield and process economics. The most important factors are alcohol type, alcohol/oil molar ratio, reaction temperature and time, catalyst type and amount and water content of the reactants (Meher *et al.*, 2006). Besides, the free fatty acid (FFA) content of the feedstock is also an important parameter to consider as it could affect the chemical reactions. High FFA (i.e. high acid value) in the feedstock would result in soap formation when alkali chemicals are used as catalysts because they react to neutralise the FFA in the oil (Vicente *et al.*, 2004). The soap formation could decrease the biodiesel yield and complicate the separation and purification of biodiesel product. The saponification can be avoided by pre-treating the oil with an acid catalyst to convert the FFAs into esters before the alkali catalyst is used. However, the acid-catalysed reaction is much slower than the alkaline-catalysed reaction (Ramadhas *et al.*, 2005). Ghadge and Rahman (2006) found that to reduce the acid value of mahua oil from 34 to less than 2 mg [KOH]g [oil]⁻¹, the optimum methanol-to-oil and H₂SO₄-to-oil ratios were 0.32 v/v and 1.24 (w/v), respectively, at the reaction temperature of 60 °C and the reaction time of 1.26 h. After this pre-treatment, they used 0.25 (v/v) methanol-to-oil and 0.7% (w/v) KOH as a catalyst for biodiesel production.

To produce biodiesel from salmon oil separated from salmon processing wastes, the biodiesel yield and the behaviour of salmon oil during transesterification reactions need to be determined. Therefore, this study was initiated to:

- (1) Develop a simple and effective method to produce biodiesel from salmon oil separated from salmon processing wastes,
- (2) Optimise the biodiesel yield in relation to chemicals used during transesterification and
- (3) Evaluate the economics of the biodiesel produced from salmon oil as compared to biodiesel produced from plant oils.

2. Materials and methods

2.1. Salmon-waste oil extraction

Two kinds of salmon oil were tested in this study. The first kind was oil separated from acidified salmon hydrolysate. The

oil was separated by centrifugation of acidified salmon hydrolysate for 20 min at 7000 rpm, with a relative centrifugal field of 7970g. The second kind was the oil separated directly after grinding of fresh salmon processing by-products. Fig. 1 shows a schematic of the unit operations involved in oil extraction and biodiesel production from both oils. Both hydrolysate and salmon oil were provided by a salmon by-product processing company in Alaska. In the following description, the first and second kinds of oil are called acidified and non-acidified oil, respectively.

2.2. Biodiesel production

Preliminary experiments were conducted on the production of biodiesel from the acidified fish oil using the one-step alkaline-catalysed transesterification process. It was found that the esters' yield was low and separation of esters was

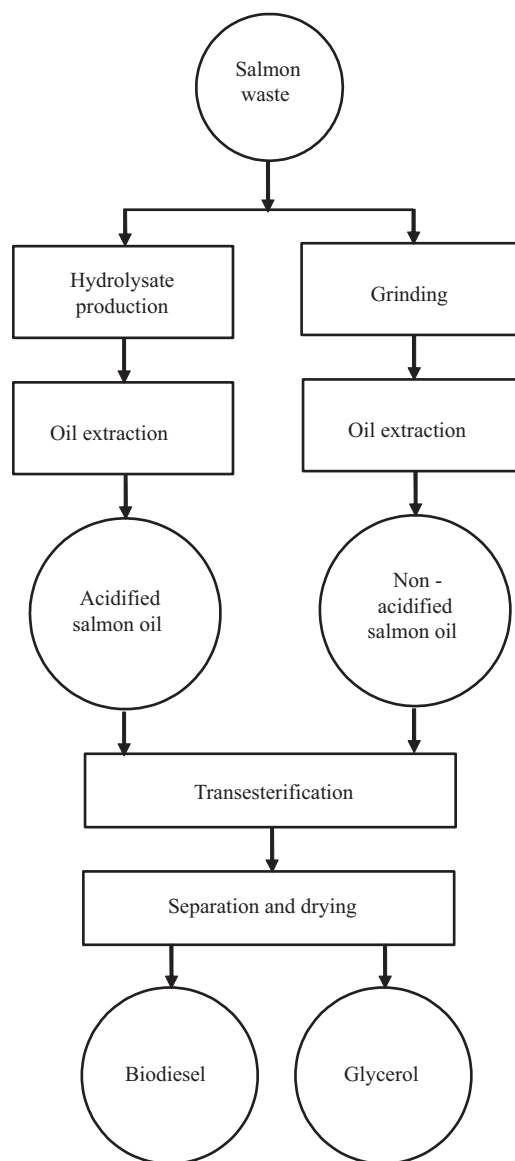


Fig. 1 – Unit operations for biodiesel production from salmon waste.

problematic due to the high acid values. Therefore, a two-step process was performed. The first step was an acid-catalysed pre-treatment to esterify the FFA and therefore reduce the acid value before the second step wherein the triglycerides were transesterified with an alkaline catalyst.

2.2.1. Experimental set-up

Glass Erlenmeyer flasks were used as batch reactors. After adding the reactants, each reactor was closed with a rubber stopper. A thermometer was placed through the stopper to monitor the temperature inside the reactor. Hot plates with magnetic stirrers were used for heating and mixing the reactor contents. Mixing was applied at 600 rpm for all experiments. According to [Vicente et al. \(1998\)](#), stirring at 600 rpm is sufficient to overcome mass transfer limitation during biodiesel production from soybean oil.

2.2.2. Acid-catalysed esterification

The main objective of acid-catalysed esterification was to reduce the acid value of the oils. The effects of different methanol/oil molar ratios (MRs) of 3.1:1, 4.7:1 and 9.1:1 on the reduction of acid value were studied using 1% (w/w) H_2SO_4 as a catalyst. Similar MRs were also applied by [Ramadhas et al. \(2005\)](#) during biodiesel production from rubber seed oil having 17% FFA. An increase in the transesterification rate was found with increasing reaction temperature. However, the maximum temperature should not exceed the boiling point of the reactant; e.g. 65 °C for methanol ([Vicente et al., 1998](#)). In this step, acidified and non-acidified oil were heated to a reaction temperature of 52 ± 2 °C. The required amounts of methanol and H_2SO_4 were added to the oil after they were heated to the same temperature. A reaction time of 1 h was used, which was selected based on the findings from [Canakci and Van Gerpen \(2001b\)](#) and [Ghadge and Raheman \(2006\)](#). The latter authors found that increasing the reaction time beyond 1 h did not have a significant effect on reducing the acid value. They attributed this to the inhibition of the reaction due to water formation during the esterification of FFA. At the end of the reaction, the content of each reactor was transferred to a separating funnel to settle for 3 h. Afterwards clear separation of different layers was noticed. According to [Ghadge and Raheman \(2006\)](#), the top layer contained mainly water and methanol and the bottom layer contained the esterified oil. The bottom layer was used in the transesterification step (i.e. second step). The effectiveness of the first step was evaluated by measuring the acid value of this intermediate product. Acid value is defined as the amount of KOH required to neutralise 1 g of fat. The yield of this step was calculated as the weight of pre-treated esterified oil divided by the weight of raw oil used.

2.2.3. Alkaline-catalysed transesterification

According to [Freedman et al. \(1986\)](#), stoichiometrically, the overall transesterification requires 3 mol of alcohol to yield 3 mol of ester and 1 mol of glycerol according to the following:



Since this reaction is reversible, in practice, the alcohol MR should be higher than the stoichiometric requirement in

order to drive the reaction towards completion. For non-acidified oil, the pre-treated (esterified) oil at an MR of 3:1 was used as the feedstock for the alkaline-catalysed transesterification at three different MRs of 3.1:1, 6.1:1 and 9.1:1, that gives total MRs in both steps of 6.2:1, 9.2:1 and 12.2:1. Each MR level was run at three levels of KOH: 0.5%, 1% and 1.5% (w/w).

Similar to the first step, the reaction was performed at 52 ± 2 °C. The required amount of KOH was dissolved into the required methanol amount. The KOH-methanol mixture was heated to the same temperature, and then added to the pre-heated-pre-treated oil. The recording of the reaction time was started as soon as the KOH-methanol mixture was added to the reactor. For each combination of MR and KOH, three reactors were used. The first reactor was used for measuring the specific gravity of the reactants at different reaction times and the other two were used to calculate the yield. After the reaction was completed, the content of the second reactor was poured into a separating funnel and allowed to separate for 4 h. Two distinctive layers were noticed: the top layer containing the methyl ester and the lower layer mainly containing glycerol and the excess of KOH and methanol. The top layer was washed with distilled water three times. The washing step removes residual KOH, methanol and soap. Then the washed esters were heated at 103 °C for 10 min to remove residual water. The product before and after drying was weighed to calculate the ester yield by dividing the final weight of ester by the initial weight of the oil.

2.3. Measurements

2.3.1. Fatty acids analysis

Fatty acid contents of non-acidified and acidified fish oil and non-acidified oil methyl esters were measured after preparing the fatty acid methyl esters (FAMES) as described by [Oliveira and Bechtel \(2006\)](#). About 15 mg of oil or methyl ester was dissolved in 1.9 ml of isooctane (Sigma grade 99%, St Louis, MO). A 100 µl solution of 10 mg [methyl tricosanoate]/ml [iso-octane] was added. Methyl tricosanoate was purchased from Nu-Chek Prep Inc. (Elysian, MN). Then 200 µl of 2N KOH in methanol was added. The contents were mixed for 60 s using a vortex mixer. The samples were centrifuged for 3 min at 3400 rpm and the lower layer was discarded. This procedure was repeated twice using 0.5 ml of a saturated solution of ammonium acetate in water, followed by 0.5 ml of de-ionised water. Methyl esters in isooctane were then dried by adding 250 mg of anhydrous sodium sulphate. The samples were then centrifuged at 3400 rpm for 20 min and the FAMES were taken for analysis.

The FAMES were analysed in duplicate using a gas chromatographer (Hewlett Packard 5890, Palo Alto, CA) equipped with a flame ionisation detector and an auto injector. A 1.0 µl sample was injected and subjected to a 1:100 split ratio. A 100 m capillary column (Supelco 2560) with a 0.25 mm inside diameter and a film thickness of 0.20 µm was used. Hydrogen was used as a carrier gas with a linear flow rate of 0.27 m s^{-1} and a head pressure of 230 kPa. Injector and detector temperatures were set at 220 °C. The column temperature was held at 75 °C for 10 min, increased to 175 °C at $20^\circ\text{C min}^{-1}$ and held for 29 min, and then increased to a final temperature of 225 °C at 5°C min^{-1} and held for 12 min.

The detector signal was integrated and quantified using Chrom Perfect Spirit version 5.5 software (Justice Laboratory Software, Denville, NJ).

2.3.2. Other measurements

The moisture content of each salmon oil used in this project was measured in duplicate as described by [Dorado et al. \(2002\)](#). Acid value was measured according to the procedure of [AOCS \(1998\)](#). The acid values of the acidified and non-acidified salmon oil were measured in duplicate before and after the pre-treatment step.

To estimate the required reaction time in the second step, the change in the specific gravity of the reactants was used, similar to the method used by [Al-Widyan and Al-Shyoukh \(2002\)](#) who evaluated the completion of acid-catalysed transesterification of palm oil. Lower values of specific gravity indicated that more of the heavy glycerine was removed and the reaction was moving towards the direction of completion. When specific gravity remained constant, the reaction was considered to be complete. Two samples were taken as duplicates from each reactor at each time of measurement. To stop the reaction, the samples were directly placed in ice and centrifuged at 5000 rpm for 5 min. A specific volume of the top layer of each sample was accurately weighed to calculate the specific gravity. The measurements of the specific gravity were done under ambient temperature (24–27 °C).

2.4. Statistical analysis

The experimental data of methyl ester yields were analysed by a two-factor ANOVA method using Microsoft® Excel software 2003 (Microsoft Corp., Seattle, WA). The differences in each of the measured parameters were compared after calculating Fisher's least square difference (LSD) at $\alpha = 0.05$. The differences between each pair of the reactors were considered significant if they were higher than the 5% LSD.

3. Results and discussion

3.1. Characterisation of salmon-waste oils

The fatty acid contents of non-acidified and acidified salmon oil are shown in [Table 1](#). As can be seen, there were no significant differences between the two oils with regard to the fatty acid profile and contents. The saturated and unsaturated fatty acids represented about 27% and 73%, respectively. The saturated fatty acids were comprised mainly of palmitic acid (C16:0), while the unsaturated acids were comprised mainly of oleic acid (C18:1). According to [Ramadhas et al. \(2005\)](#), the methyl esters of saturated fatty acids have a higher cloud point, cetane number and better biodiesel stability. Like many other biodiesels, the vulnerability of the salmon oil-biodiesel to oxidation instability could be alleviated by antioxidant additives. [Waynick \(2005\)](#) mentioned some antioxidants commonly used for stabilisation of fatty acid esters. Some of them are natural such as tocopherols and others are synthetic such as tertiary butylhydroquinone (TBHQ), pyrogallol (PY) and propyl gallate (PG).

Table 1 – Fatty acid profile and contents (g/100 g[oil]) of non-acidified and acidified salmon oil

Type	Fatty acid	Non-acidified oil	Acidified oil
Saturated	Lauric acid (C12:0)	0.14	0.15
	Tridecanoic acid (C13:0)	0.07	0.07
	Myristic acid (C14:0)	6.85	7.04
	Pentadecanoic acid (C15:0)	0.88	0.74
	Palmitic acid (C16:0)	14.83	14.95
	Margaric acid (C17:0)	0.68	0.6
	Stearic acid (C18:0)	3.12	3.71
	Total	26.57	27.26
Unsaturated	Myristoleic acid (C14:1)	0.10	0.09
	Palmitoleic acid (C16:1)	6.46	6.9
	Oleic acid (C18:1)	18.22	21.95
	Linoleic acid (C18:2)	4.88	4.44
	Linolenic acid (C18:3)	11.67	7.97
	Arachidonic acid (C20:4)	3.33	2.79
	Brassic acid (C22:2)	0.19	0.11
	EPA, Timnodonic acid (C20:5 n-3)	11.13	11.52
	C22:3 n-3	0.43	0.22
	C22:4 n-6	0.11	0.12
	DPA, Clupanodonic acid (C22:5 n-3)	3.29	3.67
	DHA,	13.63	12.96
	Docosahexaenoic acid (C22:6 n-3)		
	Total	73.43	72.74
Data are the averages of duplicate samples.			

The acid values of both oils were determined to be 12.05 and 3.47 mg [KOH] g [oil]^{−1}, respectively, for the acidified and non-acidified salmon oil, which were higher than the maximum acid value of 2 mg [KOH] g [oil]^{−1}, recommended by [Canakci and Van Gerpen \(2001b\)](#) for alkaline transesterification. The moisture contents were 0.12% and 0.07%, respectively, which are far below the values that could cause biodiesel yield decrease. [Canakci and Van Gerpen \(2001a\)](#) found that an oil moisture content of more than 0.5% resulted in ester conversion below 90%.

3.2. Acid- catalysed transesterification (pretreatment step)

The effect of MR on the acid value of the two salmon oils after the pre-treatment step is shown in [Fig. 2](#). The pre-treatment was performed using 1% H₂SO₄ as a catalyst. As can be seen, the pre-treatment of the non-acidified oil with an MR of 3.1:1 reduced the acid value from 3.5 to 2.6 mg [KOH] g [oil]^{−1}. With MRs of 4.7:1 and 9.1:1, the acid value decreased to 0.8 and

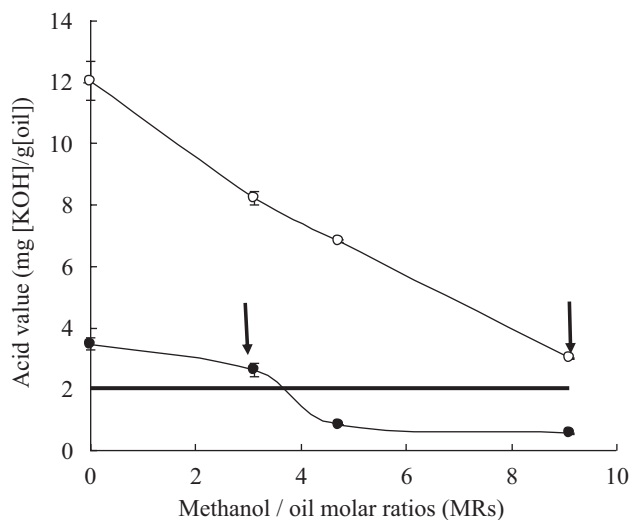


Fig. 2 – The relationship between acid value of salmon oils and MRs during acid-catalysed pre-treatment; y error bars are standard deviations; arrows show the pre-treated oil used for alkaline-catalysed transesterification: ○, acidified oil; ●, non-acidified oil; —, recommended acid value (Canakci & Van Gerpen, 2001b).

0.6 mg [KOH] g [oil]^{−1}, respectively, which are below the recommended value as shown in Fig. 2. However, a successful alkaline transesterification was achieved with the pre-treated oil with an MR of 3.1:1. Therefore, all alkaline transesterification experiments (i.e., the second step) were conducted with this MR. For the acidified salmon oil, a lower acid value of 3 mg [KOH] g [oil]^{−1} was obtained with an MR of 9.1:1. Unlike the non-acidified oil, there was a sharp decrease in the acid value of the acidified oil after the pre-treatment with an MR greater than 3.1:1. Successful alkaline transesterification was obtained at the acid value of 3 mg [KOH] g [oil]^{−1}, even though it was higher than the value recommended by Ma and Hanna (1999) and Canakci and Van Gerpen (2001b). Therefore, it may be concluded that for achieving successful alkaline transesterification, 3 mg [KOH] g [oil]^{−1} may be used as an upper limit for the acid value. This translates to 1.5% (FFA) in the fish oil. These results contradict the results of Van Gerpen (2005) who mentioned that for animal fats normally containing 5–30% of FFAs, the reaction can still be catalysed with an alkali catalyst up to 5% FFA, but additional catalyst must be used to compensate for the catalyst lost to soap. In our experiments it was not possible to obtain good separation after alkaline-catalysed transesterification without the pre-treatment step. The yield of the pre-treatment step was defined as the mass of the intermediate product divided by the mass of the raw oil. The MR had no profound effect on the yields, from the pre-treatment step, for both acidified and non-acidified oils (data not shown). Based on the weight of oil used in the pre-treatment step an average yield of about 100% could be calculated, which is similar to the yield obtained by Ramadhas *et al.* (2005) who conducted the pre-treatment of rubber seed oil that contained 17% FFA, using 0.5% H₂SO₄ and MR of 6:1.

3.3. Alkaline-catalysed transesterification (second step)

3.3.1. Non-acidified oil

The effects of different MRs and KOH levels on the specific gravities of the reactants during the alkaline-catalysed transesterification step are shown in Fig. 3. The specific gravity decreased over the reaction time and eventually reached a fairly constant value. Higher MRs resulted in lower specific gravities. Comparable results were obtained by Al-Widyan and Al-Shyyoukh (2002) during the acid transesterification of waste palm oil. Generally, despite the presence of some deviations, it can be noticed that higher KOH levels resulted in higher specific gravities. With an MR of 3.1:1, an asymptotic value for specific gravity could be obtained after a reaction time of 45 min, while with an MR of 9.1:1, an asymptotic value could be obtained after 30 min. These results may indicate that a shorter reaction time is required when higher MRs are used. However, all alkaline transesterification experiments, used for yields calculations, were carried out at a reaction time of 1 h. For rubber seed oil with 17% FFA, Ramadhas *et al.* (2005) found that a reaction time of 30 min was sufficient for the esterification in the second alkaline step. Although the change of specific gravity over the transesterification reaction time could be considered as a fast and an easy measurement to determine the reaction time required as proposed by Al-Widyan and Al-Shyyoukh (2002), research is needed to compare this parameter with the change of triglyceride content over reaction time.

The methyl esters yields at different MRs and KOH levels before the washing and drying step are shown in Fig. 4. It should be mentioned that the MRs are the total MRs used in

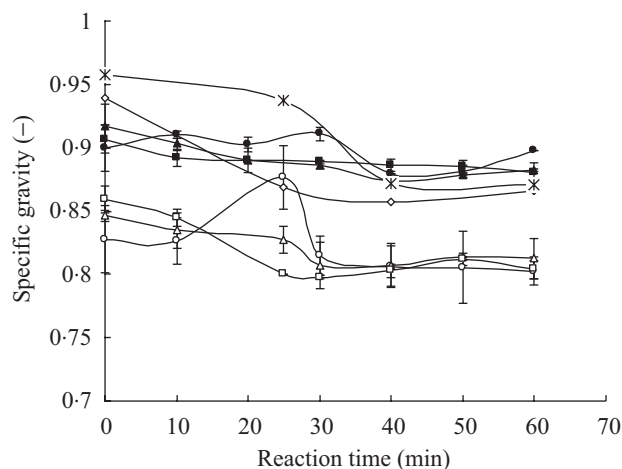


Fig. 3 – Relation between specific gravity and reaction time at different MRs and KOH levels; y error bars are the standard deviations between duplicate measurements at ambient temperature (24–27 °C): ○, MR 9.1:1 and 0.5% KOH; △, MR 9.1:1 and 1% KOH; □, MR 9.1:1 and 1.5% KOH; ●, MR 6.1:1 and 0.5% KOH; ▲, MR 6.1:1 and 1% KOH; ■, MR 6.1:1 and 1.5% KOH; ◇, MR 3.1:1 and 0.5% KOH; ✕, MR 3.1:1 and 1% KOH.

both steps. According to Ma and Hanna (1999), a better conversion could be obtained in a shorter time with higher MRs. MR and KOH level and their interaction affected the methyl esters yield. The two-factor ANOVA indicated significant differences between the studied treatment. Therefore, each pair of the treatments was compared using Fisher's least significant difference tests. At the same KOH level, there were significant differences in the unwashed methyl esters yield between 6.2:1 and 9.2:1 and between 6.2:1 and 12.2:1 MRs. However, there were no significant differences between 9.2 and 12.2 MRs. A maximum yield of about 99% was obtained with 9.2:1 MR and 0.5% KOH. The methyl esters produced were bright yellow in colour. The results of Ramadhas *et al.* (2005) on biodiesel production from rubber seed oil showed a maximum conversion at 9:1 MR and 0.5% NaOH in the second step. According to Vicente *et al.* (2004), the biodiesel weight yields relative to the initial weight of vegetable oil should be nearly 100% if there is no saponification or neutralisation of the FFA in the vegetable oil. These two reactions produce caustic soap, which could reduce the final yield. During biodiesel production from vegetable oil, Kim *et al.* (2004) found that there was no significant increase in the biodiesel yield, from vegetable oil, on increasing MR beyond 9:1. On the other hand, for the effect of KOH level, at 6.2:1 MR, an increase of KOH level significantly decreased the methyl esters' yield. At 9.2:1 MR, there was a significant difference between the reactors catalysed by 0.5% KOH and that catalysed by 1.5%, while at 12.2:1 MR; the KOH level had no significant effect on the methyl esters' yield.

The glycerol phase (i.e. mainly composed of glycerol, catalyst and remaining alcohol) yields under different conditions are shown in Fig. 5. A higher MR or KOH level resulted in a higher glycerol phase yield. This phase can undergo further processing to produce crude glycerol that can be used for manufacturing medical and industrial chemicals such as CP glycerol, USP glycerol and dynamite glycerol (Ma & Hanna, 1999; Zappi *et al.*, 2003). However, for small-scale plants

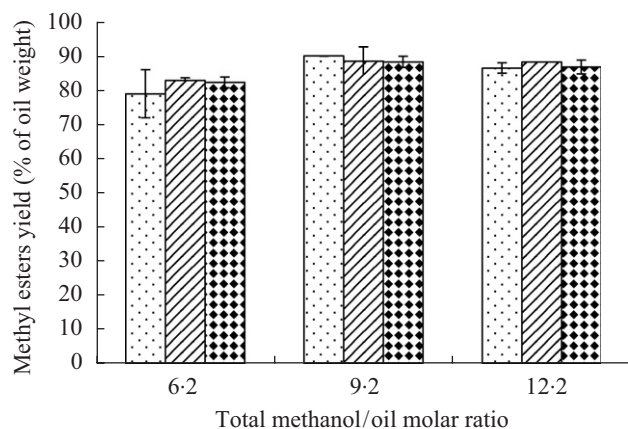


Fig. 4 – Methyl esters yield, before the washing step, at different MRs and different amounts of KOH; y error bars are standard deviations between duplicate reactors: □, KOH 0.5% (w/w); ▨, KOH 1% (w/w); ▩, KOH 1.5% (w/w).

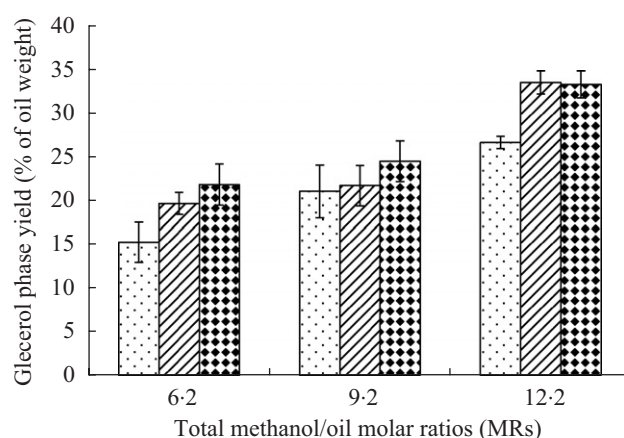


Fig. 5 – Glycerol phase yields at different MRs and KOH levels; y error bars are standard deviations between duplicate reactors: □, KOH 0.5% (w/w); ▨, KOH 1% (w/w); ▩, KOH 1.5% (w/w).

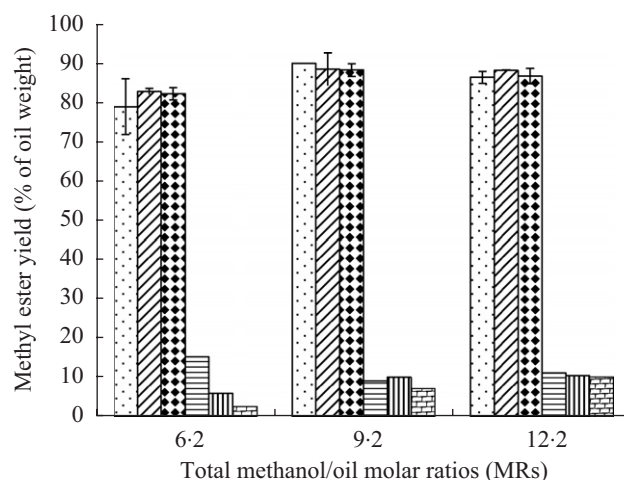


Fig. 6 – Methyl esters yield, after washing and drying, at different MRs and KOH levels; y error bars are standard deviations between duplicate reactors: □, final yield at KOH 0.5% (w/w); ▨, final yield at KOH 1% (w/w); ▩, final yield at KOH 1.5% (w/w); ▪, yield loss at KOH 0.5% (w/w); ▫, yield loss at KOH 1% (w/w); ▬, yield loss at KOH 1.5% (w/w).

wherein purification facilities are not available, this phase could be mixed with manure to increase its fertilising quality or be used as a feedstock for biogas production (Ahn *et al.*, 1995).

The methyl esters yields at different MR and KOH levels after washing three times with distilled water and drying are shown in Fig. 6. An interphase was observed during washing. It was insoluble in both ester and water and some ester was removed with it (Canakci & Van Gerpen, 2001b). A maximum average methyl esters' yield of about 90% was obtained at 9.2:1 MR and 0.5% KOH. A maximum yield loss of about 15% was obtained with 6.2:1 MR and 0.5% KOH mainly due to the formation of emulsion during washing. The yield loss was determined by dividing the methyl ester weight reduction, after washing and drying, by the methyl ester weight before

washing and drying. During the washing of ethyl ester made from rapeseed oil, Korus *et al.* (1993) found that 18% of ester was lost due to the formation of emulsion that resulted from the agitation of the ester with washing water. However, a more controlled washing and drying process could be designed in large-scale applications to reduce emulsions and avoid the yield loss. For biodiesel production from yellow grease and brown grease, Canakci and Van Gerpen (2001a) found a yield of 91% due to interphase and washing losses.

The thermal, rheological and stability properties of the salmon oil methyl esters were evaluated and reported by Chiou *et al.* (2006). Despite the difference in the composition of salmon oil from that of corn oil, salmon oil methyl esters had comparable viscosity, volatility, low temperature properties, heating value, acid value, and specific gravity and better oxidative stability than that of corn oil methyl esters.

3.3.2. Acidified oil

In a preliminary experiment, the acidified oil was transesterified with one alkaline-catalysed step using 1% KOH as the catalyst. The results showed poor separation giving a methyl esters yield of about 22%. The salmon hydrolysate contained formic acid and the fish oil separated from the hydrolysate had a pH of about 3.7. Afterwards an experimental run was carried out in a two-step process. In the first step, methanol was added without H_2SO_4 and then the second step was conducted using KOH as a catalyst. The results showed poor separation as similarly obtained in one-step alkaline catalysed (data not shown). The two-step procedure was revised to include H_2SO_4 in the first step, similar to the procedure used for the non-acidified oil. The results showed that a methyl ester yield of about 97% could be obtained (Table 2). A three-step process was also attempted in an effort to improve the biodiesel yield, in which the acidified oil was first treated with methanol only, and then H_2SO_4 was added as the catalyst and followed by an alkaline-catalysed step. All the experiments were carried out in duplicate at $52 \pm 2^\circ C$. The amounts of the chemicals used and the biodiesel yields from these experiments are shown in Table 2. It can be seen that there was no significant effect on the biodiesel yield between two- and three-step processes. An average biodiesel yield of 97.6% was obtained. The biodiesel produced was reddish orange in colour. It could be concluded that formic acid added during hydrolysate production did not act as a catalyst in the pre-treatment step. The two-step process is recommended over the three-step process because the latter requires more energy and effort without conferring any benefits.

3.4. Cost analysis

A preliminary economic analysis was performed to compare the costs of salmon oil biodiesel with that of soya bean oil biodiesel. Haas *et al.* (2006) presented a computer model to estimate the capital and operating costs for a soybean biodiesel plant with an annual capacity of 38,000 m³. Assuming that the only difference between fish oil and soybean oil biodiesel plants hardware would be the costs of raw materials used for biodiesel production (salmon oil, methanol, KOH and

H_2SO_4), a similar cost analysis was carried out for the production of biodiesel from salmon oil. The results of this analysis and that of Haas *et al.* (2006) are shown in Table 3. For both biodiesels the most important factor affecting biodiesel cost is the price of the feedstock. The biodiesel production costs of salmon oil and soybean oil were estimated to be 1.07 and 0.53 US \$/l, respectively. Such a huge difference is due to the high price of salmon oil at 950 US\$/ton as used for industrial applications. However, substantial reduction in the salmon oil biodiesel costs would be expected when there was no alternative use of the non-edible salmon oil.

Table 2 – Biodiesel yield from acidified salmon oil

	One-step process	Two-step process	Three-step process
<i>First step</i>			
MR		8.95	5.85
H_2SO_4 , % (w/w)		1	
Reaction time, h		1	1
<i>Second step</i>			
MR	10.3	4.7	2.84
KOH, % (w/w)	1	0.8	–
H_2SO_4 , % (w/w)	–	–	0.9
Reaction time, h	1	0.5	1
<i>Third step</i>			
MR			4.84
KOH, % (w/w)			0.8
Reaction time, h			0.5
Total MR	10.3	13.65	13.53
Biodiesel yield, % of oil	22	97.6 ± 0.2	97.6 ± 1.5
Glycerol yield, % of oil		17.7	17.1
Data are the averages of duplicate experiments.			

Table 3 – Costs of salmon biodiesel as compared with soybean biodiesel

Cost components	Salmon biodiesel, US\$/l	Soybean biodiesel, US\$/l (Haas <i>et al.</i> , 2006)
Total raw material	1.056	0.5
Utilities	0.011	0.011
Labour	0.013	0.013
Supplies (operating and maintenance)	0.004	0.004
General work (administration, taxes and insurance)	0.003	0.003
Depreciation	0.03	0.03
Total costs	1.116	0.561
Glycerol credit	0.052	0.034
Gross operating cost	1.065	0.527

4. Conclusions

One-step alkaline-catalysed transesterification was found to be ineffective for biodiesel production from salmon oils extracted from fresh salmon by-products and acidified salmon hydrolysate. This is due to the high acid value of the oils, which were measured to be 3.5 and 12.0 mg [KOH] g [oil]⁻¹, respectively, for the two salmon oils. A two-step process was found to be effective, in which salmon oil was firstly pre-treated with an acid-catalysed reaction yielding esters and reducing the acid value and then followed by transesterification using KOH as the catalyst. The 1% H₂SO₄ was used as the acid catalyst in the first step and different MRs and KOH levels were tested in the second step. The formic acid used in the production of salmon hydrolysate did not act as a catalyst in the pre-treatment step for the production of biodiesel from salmon oil. A successful alkaline-catalysed transesterification could be achieved with an acid-pre-treated salmon oil having an acid value 3 mg [KOH] g [oil]⁻¹. Statistical analysis showed significant effects of both MR and KOH levels on the biodiesel yields. The results show that a maximum yield of about 99% of the oil weight was obtained with an MR of 9.2:1 and KOH of 0.5%. The cost of salmon oil biodiesel was almost twice that of soybean oil biodiesel due to the high price of the salmon oil biodiesel. However, substantial reduction in the salmon biodiesel costs would be expected when there was no alternative use of the non-edible salmon oil.

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